SOME EFFECTS OF HIGH PRESSURE ON DEVELOPING MARINE FORMS

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The action of hydrostatic pressure, below certain limits, in producing an increase in the contractility of cardiac and skeletal muscle, raises a question of prime interest as to the nature of the effect. Does an increase in the absolute pressure of the environment of a tissue, with the resulting increase in tissue density, give rise to a general stimulation of the fundamental processes in the cells? Several lines of approach have been considered for an answer to this question, but the aim in the present experiments will be to deal only with the influence of pressure on certain of the fundamental processes in the early development of marine eggs.

The experimental observations relate mainly to two features: first, the effect of maintained compression on the rate of development of fertilized eggs, particularly in the early stages; and second, the effects produced by pressure on the rate of the heart in embryos at the stage when pulsations are just beginning and then later when the rhythm is fully established.

The observations were made in an apparatus having essentially the same construction as that previously described (Edwards and Cattell, 1928). The fertilized eggs of *Fundulus* were placed in glass vials filled with sea water and covered with thin rubber membrane to prevent their escape. One lot of the eggs was then placed in the compression chamber, which was also filled with sea water, and another lot, the control sample, was placed in a second chamber similar to the first. In this manner the factors of temperature, respiration and amount of agitation were kept constant, and the experimental sample differed only in having pressure applied for known intervals.

In a few experiments the technic was modified to permit observation of the eggs during the period when they were under the action of pressure. The essential things for this purpose consisted of a heavy glass window mounted in one end of the compression chamber, which made it possible to view with low magnification objects placed immediately beneath it, and a depression slide fixed in position close to the inside surface of the window to contain the eggs. A small mirror backing the slide and a " Pointolite " lamp, which directed a beam of light through the window and against the mirror, permitted fair illumination for a microscopic examination of the eggs in the depression of the slide. All

of the observations on the change of heart rate under pressure were made by using this adaptation of the pressure apparatus.

An observation of general interest arising from these experiments on compression of the developing egg is the extremely slight change that occurs in the egg structures when subjected to comparatively high pressures. The method has permitted observations on the diameter of the egg, the size and state of aggregation of the fat globules, the size and position of the blastodisc, and the size of the finer blood vessels in parts of the embryo. A close study of these different parts of the developing egg, made during and immediately after the onset of pressure of 110 atmospheres, reveals no significant changes. Observations on advanced embryos within the egg, made while pressures of 1500 pounds per square inch were applied, may reveal nothing more than a few quick

TABLE I

The effect of pressure on the cleavage of Fundulus eggs. The time intervals in the fourth column signify the delay in the development of eggs subjected to pressure as compared with those maintained under control conditions. The observations were made at the cleavage stage shown in the fifth column.

Experiment No.	Pressure	Duration of compression	Delay of pressure eggs	Stage of cleavage
	lb.	*min.*	*min.*	
1	1500	140	15	second
2	1700	60	15	first
			13	second
3	1500	108	20	third
4	1950	120	10	third
5	1950	100	15	first
			15	second

movements of the embryo not unlike those shown at indifferent intervals by material of this kind.

In order to test the effects of pressure on the rate of cell division it was necessary to have some standard for comparing the eggs subjected to pressure with those maintained under control conditions. In our initial experiments the completion of the membrane between two daughter cells was taken arbitrarily as an end-point. With this criterion the data contained in Table I show that the eggs subjected to pressure are delayed about fifteen minutes in reaching a given stage of development. The exact time, however, at which a batch of developing eggs reached a given stage in division was often difficult to determine. Additional observations, therefore, were taken (1) by making counts on the control

and the experimental samples to determine the predominant stage at a given time; and (2) by removing samples of 25 or more eggs which were placed in a fixing solution for later examination. The results by these methods of study are set forth in detail in Tables II and III and the evidence they bring forth lends support definitely to the conclusion that compression retards cell division.

The eggs subjected to pressure have been carefully watched for any abnormalities that might occur. A number of monsters have been found of the types showing gross distortions of the body, changes of the cardiovascular system, and tendencies towards anophthalmus. The percentage of abnormal specimens, however, was not large and there was no dominant type of dysmorphism. The abnormalities of the eye were usu-

TABLE II

The effect of pressure on the cleavage of Fundulus eggs. The data contained in the fourth and fifth columns represent simultaneous observations on samples of eggs subjected to pressure (pressure sample) and samples maintained for the control. The observations were made on living material.

Experiment No.	Pressure	Duration of compression	Pressure sample	Control sample
	lb.	*min.*	*division stage*	*division stage*
1	1300	68	1-cell	2-cell
2	1700	112	2-cell	4-cell
3	1650	60	1 and 2-cell	2 and 4-cell
4	1650	30	1-cell	2-cell
5	1575	180	2-cell	4-cell
6	1560	130	1-cell	2-cell
7	1525	140	1-cell	2-cell
8	1300	70	1-cell	2-cell
9	1500	108	2-cell	4-cell
10	1950	120	4-cell	8-cell

ally those showing one located craniad to the other but not exactly in the midline, while the deformities of the cardiovascular system appeared usually as an asymmetrical development of the vessels. The failure of the blood vascular system to develop symmetrically causes the heart to be drawn to one side of the pericardial cavity.

The *Fundulus* embryo is a favorable object for the study of the rate of the heart, since the pulsations of this organ may be observed almost at the time automaticity starts and may be followed until a completely functioning circulatory system is established. As heart automaticity is a fundamental property in development, with a fairly definite time of appearance, the study of the influence of pressure on this phenomenon presented features of unusual interest. The results of 17 experiments

are summarized in Table IV. These data show that a pressure of 1200 pounds produces a reduction in the heart rate within two minutes, amounting to an average decrease of 9.9 per cent from the control rate. The decline in rate continues, however, somewhat more slowly than the initial change, so that at the end of a 10-minute period of compression the average reduction was only 16.6 per cent below the control value. When pressure was applied, the pulsations of the heart showed a definite reduction in the rate within a half minute of the onset. Although in some instances a more or less gradual decline prevails under pressure, the more common type of change appears to be a fairly abrupt slowing.

The action of different amounts of pressure was tested, within the range of 400 to 1200 pounds, in an attempt to determine if critical points

TABLE III

The effect of pressure on the cleavage of Fundulus eggs. The data contained in the fourth and fifth columns represent counts made on samples of eggs subjected to pressure (pressure sample) and samples maintained for the control. The observations were made on fixed material.

Experiment	Pressure	Duration of compression	Pressure sample			Control sample		
			2-cell stage	4-cell stage	8-cell stage	2-cell stage	4-cell stage	8-cell stage
	lb.	*min.*						
1	1500	135	32	1	0	0	35	0
2	1500	135	30	0	0	0	27	0
3	1500	190	20	0	0	0	20	0
4	1500	175	0	28	4	0	0	32
5	1500	140	13	0	0	0	12	0
Total count			96	29	4	0	94	32

exist in the pressure effect. The results support the view that the pressure effect becomes progressively greater with the higher grades of compression on the heart. With pressures ranging from 1200 to 1900 pounds we have been able to suppress the automaticity of the heart to the extent of not being able to observe under low magnification any indication of a contractile response. We do not overlook the fact that localized fine pulsations of a few fibers may have persisted in these preparations, so small indeed as to have been beyond our range of identification, but the essential fact is that automaticity was practically stopped. On release of pressure these hearts which have been held quiescent for several minutes immediately show signs of activity and

eventually recover, thereby confirming our previous observations (Edwards and Cattell, 1928) that the pressure effect is freely reversible in nature. In establishing a pressure standstill of an embryo heart several changes have been observed to occur, such as arrhythmia, partial and complete heart block, and fibrillary motion of the auricles terminating in a cessation of activity in the region of the sinus. With the reëstablishment of activity after release of pressure, sometimes recovery appeared almost simultaneously in sinus, auricle, and ventricle, while in

TABLE IV

Effect of Pressure on the Heart Rate in Fundulus Embryo

Experiment	Age	Control heart rate	Pressure	Heart rate during compression		Heart rate after pressure release		Per cent Δ in heart rate during compression	
				2 min.	10 min.	2 min.	5 min.	2 min.	10 min.
	hr.	*beats per min.*	*lb.*	*beats per min.*	*beats per min.*	*beats per min.*	*beats per min.*		
2	120	68	1200	65	65	60	60	7.3	7.3
3	79	62	1200	58	52	53	—	6.4	16.2
4	96	55	1200	47	43	53	50	9.1	21.9
5a	119	53	1200	48	48	55	54	9.4	9.4
5b	120	55	1200	50	48	—	—	9.1	12.8
6	76	55	1200	50	50	—	—	9.1	9.1
7	121	50	1200	44	46	54	—	12.0	8.0
8	192	62	1200	53	53	62	63	14.5	14.5
9	172	57	1200	54	53	56	—	5.2	7.0
10a	216	65	1200	60	59	67	66	7.7	9.2
10b	217	66	1200	60	—	67	67	9.0	—
11	150	73	1200	67	66	72	—	8.2	9.6
17	74	72	1200	69	63	69	72	4.1	12.6
19	26	60	1200	56	50	53	60	6.6	11.7
20	120	60	1200	52	53	64	60	13.3	11.7
21	144	116	1200	96	100	106	103	17.2	13.8
22	144	112	1200	88	84	99	104	21.4	25.1
Average	....	.....	.....	.....	.....	.....	.....	9.9	16.6

other instances the return followed the reverse order of the disappearance with some degree of arrhythmia preceding the dominance of the normal type.

The remarkable effect of pressure in reducing and abolishing the rhythmicity of the embryo heart raises a question as to the nature of the action. A factor that immediately occurs to one as a possible cause for the restraining action of pressure is a direct excitation of inhibitory nerve fibers supplying the heart. Experiments designed to throw light

on this suggestion were carried out in the following way. Embryos ranging in age from 14 to 20 days were carefully dissected and the heart completely isolated from the surrounding tissue. The preparations were kept immersed in a 40/60 dilution of sea water and tap water to which was added 5 per cent of glucose. These hearts are not easy to handle on account of the very small size, but with care it was possible to mount them on a depression slide and to place them in the compression chamber where their activity could be followed through the window with a low power objective.

The results of these observations confirm in all essentials those obtained with the entire embryos. Moreover, the same type of changes was present, as, for example, the arrhythmia, the different degrees of block in conduction, and the localized areas of rhythmicity. When these results are considered in conjunction with those on whole embryos taken at an early stage before nerve connections are established, they furnish additional support to the view that the depressing action of pressure on rhythmicity is not through an effect on the inhibitory nerve mechanism.

Discussion

In the agent pressure we have an instrument by which the contractility of cardiac and skeletal muscle tissue may be greatly stepped-up, but this action, remarkable as it is for these tissues, appears to be a peculiar effect on the contractile mechanism of these types of muscle. In the present experiments we have evidence that such fundamental biological properties as cell division and automaticity of the embryonic heart become restrained by pressure—a fact that presents equally difficult questions to answer as those given by the augmentation phenomenon in certain types of contractile tissue.

While the types of chemical change underlying the processes of cell division and heart rhythmicity obviously are complex and the factors common to both cannot be set down, yet the fact is not without interest that some forms of chemical reactions are known to be influenced by pressure. Rothmund (1896) found that the acid inversion of cane sugar, a first order catalytic reaction, is decreased in velocity about five per cent when subjected to a pressure of 500 atmospheres, and on theoretical grounds there is reason to conclude, according to Jones (1915), that the velocity of second order reactions is influenced in direct proportion to the pressure.

Attention was called in the early part of this paper to the observation that the cell constituents gave no gross signs of change under pressure.

With albumin, however, an addition of energy to the material produces a tendency to coagulation, as shown by Fermau and Pauli (1915) on irradiating with radium salts, and by Bridgman (1914) with high pressures; therefore it is probable that some alteration takes place in the egg substance even at the comparatively low pressures used in our experiments, but its character is such as to be not easily recognizable. The observations of Heilbrunn (1920, 1921) show in a definite way how great are the changes in viscosity of egg protoplasm during the process of division and they emphasize also the manner in which mitosis may be inhibited by factors that tend to modify the viscosity changes. The pressure effect in retarding cell division may be, therefore, largely one of altering the viscous properties of the cell constituents.

Perhaps the one factor upon which attention first becomes focused in the attempt to account for the decrease in division rate of eggs under pressure is a possible change in the oxygen supply. This feature received thoughtful attention at the outset of our experiments, and we believe that the details of procedure employed provide adequate controls and successfully rule out a change in gas tension as a factor in our results.

Our early observations on the effect of pressure gave evidence to support the view that the rhythmicity of the isolated heart is accelerated when a compression up to 60 atmospheres (882 pounds per square inch) is made to act upon this organ. The procedure in these initial experiments, however, of subjecting the preparation to pressure for only brief intervals and of taking records of the heart cycles immediately following the onset of pressure gave rise to an incomplete conception of the action of this agent. A review of our original tracings and evidence from many additional observations made since show quite conclusively that pressure does not induce an acceleration of the rate of the heart except as a temporary event. The present results, therefore, showing always depression of the rate under pressure seem at first discordant with previous findings, but full consideration of the facts indicates quite clearly that we are dealing with the secondary action of pressure on rhythmicity in contrast to the initial temporary effect that occurred during the first eight to ten cycles in a rhythmic preparation following the onset of compression.

The effect of pressure in slowing the heart rate raises a question concerning the mechanism of this action. While a mechanical factor may have contributed some part in this type of response, it is evident that such an effect does not arise from an increase in the resistance to the

circulation by pressure narrowing the peripheral vessels, since careful observations of the size of certain small vascular channels reveal no detectable alterations in their caliber with the application of pressure. The property of automaticity of the heart, on the other hand, is influenced in a marked degree by changes in the ionic relations of its environment, and the observations of Bogojawlensky and Tammann (1898) on the conductivity-pressure coefficient of some conducting systems gives proof of the changes in the mobility of ions and of the alterations in the ionization of electrolytes by pressure. The precise changes in ionic equilibrium that may have operated in producing a retardation of the heart rate under compression cannot be given from the data at hand, but that a factor of this nature plays a part is strongly suggested by the type of effect that pressure gives in slowing the embryonic heart.

Summary

Hydrostatic pressure of as much as 1900 pounds per square inch applied to living eggs of the *Fundulus* causes no evident changes in the cell constituents.

The rate of cell division of *Fundulus* eggs was slowed by maintaining them under pressures of 1300 to 1900 pounds for periods ranging from 30 minutes to three hours. In the pressure samples a few instances appeared of abnormal forms of development.

The automaticity of the heart in young embryos becomes slowed by pressure and apparently may be abolished if the compression is maintained but returns within a few minutes following release of the pressure. In ten experiments an average decrease of 9.9 per cent in the heart rhythm under pressure was present within an interval of two minutes. The embryonic heart under pressure develops arrhythmia, types of local block, and isolated fibrillary activity.

The action of pressure in slowing the heart rate does not depend on the presence of inhibitory nerves to this organ, since compression produces a slower rhythm of (*a*) hearts from older embryos that have been dissected free of all extrinsic nerve connections; and (*b*) hearts of young embryos that have no intrinsic nerves developed.

The bearing of viscosity and ionic changes produced by pressure are considered in relation to the decrease in the rate of cell division and the automaticity of the embryonic heart.

BIBLIOGRAPHY

Bogojawlensky, A., and G. Tammann, 1898. *Zeitschr. f. phys. Chem.*, **27**: 457.
Bridgman, P. W., 1914. *Jour. Biol. Chem.*, **19**: 511.
Edwards, D. J., and McKeen Cattell, 1928. *Am. Jour. Physiol.*, **84**: 472.
Fermau, A., and W. Pauli, 1915. *Biochem. Zeitschr.*, **70**: 426.
Heilbrunn, L. V., 1920. *Biol. Bull.*, **39**: 307.
Heilbrunn, L. V., 1921. *Jour. Exper. Zoöl.*, **34**: 417.
Jones, H. C., 1915. Elements of Physical Chemistry, 4th ed. New York.
Rothmund, V., 1896. *Zeitschr. f. phys. Chem.*, **20**: 168.